9. Dry completely to remove organics and excess base.
10. Reconstitute in 10 [□]μL of 0.1% TFA (acidic).

However, the method according to the invention will only require 4 steps, summarized as follows:

1. Concentrate peptides from in-gel digest to about 20 μL.
2. Add DIEA, guanidinate lysine side-chains overnight at RT.
3. Add water-compatible sulfonation agent, 30 minutes RT.
4. Add HCl, cleanup on a C_{18} [□]μZipTip™.

Please amend the paragraph at page 17, lines 4-12, as follows:

Figure 2A-B illustrate the stability of NHS-esters according to the invention. More specifically, Fig 2A shows the stability of 3-sulfopropionic acid NHS-ester in D_2O while Fig 2B shows the stability of 2-sulfobenzoic acid NHS-ester in D_2O. The analysis was conducted on a 270 MHz NMR-instrument from JEOL. NHS-ester were put in a NMR-tube and diluted with D_2O to 700[□]μl. A single-pulse ^{1}H-NMR was conducted and the spectra analysed. The hydrolysis being measured by the ratio of the integration of the signal at 2,92 ppm for 3-sulfopropionic acid N-hyrdoxysuccinimide , 3,01 ppm 2-sulfobenzoic acid N-hydroxysuccinimide and the signals of the protons of N-hydroxysucccinimide 2,76 ppm.

Please amend the paragraph at page 18, lines 25-29, as follows:

Figure 10 shows a reflectron spectrum (positive mode, showing average masses, after filtration, smoothing 5) of the same 2-D sample as in figure 9 (remaining 95%), but after N-terminal derivatization with NHS-ester. The sample was cleaned up on a [□]μC_{18}Zip Tip, and derivatized according the protocol. The peptide *m/z* 1791 (previous figure) was quantitatively derivatized and is here observed with the extra mass of the label, *m/z* 1927.

Please amend the paragraph at page 20, lines 29-30, as follows:

10 mg of NHS-ester were put in a NMR-tube and diluted with $CDCl_3$ to 700[□]µl. A single-pulse ^{1}H-NMR was conducted and the spectra analysed. The analysis was conducted in

Please amend the paragraph at page 21, lines 7-10, as follows:

Melting point determination:

The melting point for the NHS-ester crystals was obtained on a BÛCHI Melting Point B-540 apparatus. A few crystals were put in a vial and heated until they melted. The temperature interval was from 160[□]°C to 185[□]°C and the temperature gradient 1[□]°C/min.

Please amend the paragraph at page 21, lines 12-15, as follows:

Stability test in water

10 mg of NHS-ester were put in a NMR-tube and 700[□]µl of D_2O was added. A single-pulse ^{1}H-NMR was conducted and the spectra analysed. The same sample was stored at RT (20-25 [□]°C) and after 5 and 24 hours another ^{1}H-NMR spectra were collected.

Please amend the paragraph at page 21, lines 17-21, as follows:

Stability test in air:

10mg of NHS-ester were put in a NMR Tube and analysed as above with Chloroform-D as solvent. About 100 mg of the NHS-ester were then put in a flask and kept without lid in air and RT (20-25[□]°C) for some days. The hydrolysis of the ester was followed with NMR.

Please amend the paragraph at page 22, lines 1-12, as follows:

A 3-necked roundbottomed flask (500ml) was equipped with a thermometer, dropping funnel and a degassing pipe. A gas-trap with two security-flasks (coupled in series after each other), the last containing 25% KOH-solution was fitted to the pipe During the reaction a nitrogen-balloon kept an inert atmosphere through the system. Acetic acid (70ml) and hydrogen peroxide (70g, 30% aqueous solution, 620mmol) were put in the flask and the solution was heated under stirring to 50[□]°C on a waterbath. 3-Mercaptopropanoic acid (8,20ml, 94mmol) was added very carefully through the dropping funnel over a period of about 1 hour. An exothermic reaction started at once and the temperature rose to about 80[□]°C. The solution was then cooled on an ethanol/CO_2 bath (-72[□]°C) until the temperature was again 50 [□]°C, this procedure was repeated until all the 3-mercaptopropanoic acid had been added from the dropping funnel. The reaction was then left stirring at 50 [□]°C for two hours and at RT over night.

Please amend the paragraph at page 22, lines 14-18, as follows:

The solvent was evaporated on a rotaryevaporator (water-bath 40[□]°C, 100 mbar) until the volume had been reduced to about 30ml, the rest was then removed by azeotropic evaporation with 3x300ml heptane. The resulting oil was dried in a desiccator under high vacuum over night. The crude product was a white precipitate in an oil. The yield was about 50%, estimated from the NMR-spectrum, see Fig 1.

Please amend the paragraph at page 23, lines 19-20, as follows:

All equipment that was used was dried in an oven (100[□]°C) and put in a desiccator before the synthesis.

Please amend the paragraph at page 24, lines 1-7, as follows:

the product was dried in a vacuum oven over night (RT, 1 mbar). The resulting crystals were dissolved in the minimum amount of warm EtOAc/MeOH (9:1). When everything had dissolved the solution was left to cool in RT for about three hours and then in the freezer over night. During the night white crystals had formed which were filtered on a glass filter (p3) and washed with cold ethyl acetate (5C[□]°). Finally the crystals were dried under high vacuum in a desiccator to get the DIEA-salt of the NHS-ester as white crystals (42% yield).

Please amend the paragraph at page 24, lines 12-20, as follows:

The synthesis was quite simple and gave the crude 3-sulfopropionic acid as a white slurry. The tricky part was to keep the reaction at 50[□]°C, this was done with alternating ice-bath and oil-bath which perhaps is not the most effective way. The temperature during the reaction varied from 20 [□]°C up to 80[□]°C. If a better temperature control could be maintained under the reaction maybe the yield would improve. No further purification was done since it was not necessary for the next step (synthesis of the anhydride) making the yield very hard to calculate. On the NMR-spectra you could see at least one bi-product and maybe some of the starting material (see NMR-analysis) an estimation of the purity would be around 50%.

Please amend the paragraph at page 25, lines 20-23, as follows:

The melting point of the crude NHS-ester/DIEA-salt was between 145-155[□]°C. After recrystallisation however the melting point was determined to 176-178[□]°C. This higher and much sharper melting point after purification indicates that the product has indeed become purer.

Please amend the first line of Table 1 on page 26 as follows:

Proton number	Shift ([□]δppm)	Interpretation	Group

Please amend the paragraph at page 26, lines 4-6, as follows:

The spectra also contained some by-product and some starting material giving some peaks at [□]δ2.78, [□]δ2.85, [□]δ3.18 and at [□]δ3.52. This was expected when no purification had been done.

Please amend the first line of Table 2 on page 26 as follows:

Proton number	Shift ([□]δppm)	Interpretation	Group

Please amend the first line of Table 3 on page 26 as follows:

Carbon number	shift ([□]δppm)	Interpretation	Group

Please amend the first line of Table 4 on page 27 as follows:

Proton number	Shift ([□]δppm)	Interpretation	Group

Please amend the paragraph at page 27, lines 11-15, as follows:

Typical inpurities in the crude product are NHS and DIEA. NHS gives a peak at [□]δ2.68(s) and DIEA gives peaks at almost the same ppm as seen above in the table. This makes the DIEA impurity harder to spot than NHS but it can be estimated by looking at the integral of the peaks. If there are any solvent left the MeOH gives a peak at [□]δ3.49(s), EtOAc at [□]δ2.05(s), [□]δ1.26(t) and at [□]δ4.12(q) and finally DCM at [□]δ5.30(s).

Please amend the paragraph at page 28, lines 20-25, as follows:

Fraction 1 was dissolved in MQ (11.098 ml, 100 mg/ml), filtered and used 3X1 ml in reversed phase preparative HPLC; Column: Supelcosil LC-18, 10 cm X 21.2 mm, 2[□]μ; Flow: 10 ml/min, Method: 0-10 min. isocratic 5% acetonitrile containing 0.1 % TFA B in water, 2 min. sample injection, 10-15 min. Gradient 5-12 % B in water. The fractions were evaporated and freeze dried to give a white solid/transparent viscous oil (totally 237.7 mg) of not purified product in DIEA salt form, NHS, DIEA and side product. A

Please amend the paragraph at page 29, lines 1-7, as follows:

previous more successful attempt using reversed phase preparative HPLC with the same column and system but another method: 0-6 min. isocratic 5 % acetonitrile containing 0.1 % TFA B in water, 2 min. sample injection, 6-18 min. Gradient 5-25% B in water, resulted in the product as a DIEA salt with approximately 5% NHS left and some traces from side-product in the aromatic area.
H^1 NMR (D_2O) [□]δ:8.0-8.1 (dd, 1H) 7.9-8.0 (dd, 1H) 7.7-7.8 (m, 2H) 3.6-3.8 (m, 2H) 3.1-3.2 (m, 2H) 3.0 (s, 4H) 1.2-1.3 (m, 15 H) and 2.7 (s, 0.2 H, NHS peak).

Please amend the paragraph at page 29, lines 9-14, as follows:

Acetone (2.5 ml cold, 0^0C, ice-water bath) was added to fraction 2 dropwise to give a white precipitation after 20 min. in room temperature and 25 min. in 4^0C. The precipitate was filtered and washed carefully in acetone (24 ml cold, 00C, ice-water bath) to give the product as a DIEA salt (612.7 mg, 46.3 %).
H^1 NMR (D_2O) [□]δ:8.0-8.1 (dd, 1H) 7.9-8.0 (dd, 1H) 7.7-7.8 (m, 2H) 3.6-3.8 (m, 2H) 3.1-3.3 (m, 2H) 3.0 (s, 4H) 1.2-1.3 (m, 15 H).

Please amend the paragraph at page 30, lines 12-21, as follows:

Model peptides and tryptic digests of various proteins were dissolved in about 20 [□]μL of base which was prepared by mixing deionized water with diisopropylethylamine (DIEA) in the ratio of 19:1 v:v. Peptide mixtures from in-gel digests were concentrated to a final volume of about 20 [□]μL and 1 [□]μL of DIEA was added to make the solution basic. 5 [□]μL of sulfonic acid active ester reagent at 100 mg/mL is added and the solution vortexed. The pH of each reaction is checked to ensure that it is still basic and adjusted if necessary. The reaction is allowed to proceed for 30 min. at RT. The samples are acidified with 5 [□]μL of 1 N HCl and cleaned up directly using C_{18} mini-columns ([□]μC_{18} Zip Tips[□]™, Millipore, Bedford MA). The sulfonated peptides were eluted from the columns in 4-20 [□]μL of acetonitrile:H_2O (1:1 v:v) containing 0.1% TFA.

Please amend the paragraph at page 30, lines 27-30, as follows:

Model peptides and tryptic digests of various proteins were dissolved in about 20 [□]μL of base which was prepared by mixing deionized water with diisopropylethylamine (DIEA) in the ratio of 19:1 v:v. Peptide mixtures from in-gel digests were concentrated to a final volume of about 20 [□]μL and 1 [□]μL of DIEA was added to make the solution basic. Two-

Please amend the paragraph at page 31, lines 1-11, as follows:

[□]μL of aqueous 0.5 M O-methylisourea hydrogensulfate was added and the solutions were vortexed. The pH of each solution was checked, and adjusted if necessary, to insure that they were still basic after addition of the guanidination reagent. The reactions were then allowed to proceed at room temperature (RT) for varying lengths of time (a few hours to two days). Typically, the room temperature reactions were allowed to proceed overnight. In the morning, 5 [□]μL of sulfonic acid active ester reagent at 100 mg/mL is added and the solution vortexed. The pH of each reaction is checked to ensure that it is still basic and adjusted if necessary. The reaction is allowed to proceed for 30 min. at RT. The samples are acidified with 5 [□]μL of 1 N HCl and cleaned up directly using C_{18} mini-columns ([□]μC_{18} Zip Tips[□]™, Millipore, Bedford MA). The guanidinated-sulfonated peptides were eluted from the columns in 4-20 [□]μL of acetonitrile:H_2O (1:1 v:v) containing 0.1% TFA.

Please amend the paragraph at page 34, lines 4-13, as follows:

Acylation with vinyl-pyridine: The lyophilised protein (2.4 mg) was dissolved in 800[□]μl of a buffer solution consisting of 8M urea, 50mM Tris-HCl pH 8.0 and 50mM DTT and incubated at 30°C for 30 min. 10[□]μl 4-vinyl pyridine was added (to prevent formation of disulphide bonds) and the sample was incubated for another 1h at 30°C. The sample was desalted using a NAP-10 column (Amersham Pharmacia Biotech), equilibrated with 100mM NH_4HCO_2, pH8.8 and eluted in 1.2 ml.
The sample was digested with trypsin (Promega), 1[□]μg trypsin/100[□]μg protein, for 6h at 30°C and the reaction was stopped by the addition of TFA to a final concentration of 1%. The digest was diluted in 50% AcN:0.5% TFA to a final concentration of 100ng/[□]μl (1.5pmol/[□]μl).

Please amend the paragraph at page 34, lines 15-24, as follows:

N-terminal derivatization with NHS-ester of 3-Sulfopropionic acid anhydride: Tryptic digest of 4VP-BSA (3pmole) were dried on a speed vac and reconstituted in 10[□]µl of deionized H_2O:diisopropylethylamine (19:1, v:v). The NHS-ester was dissolved in de-ionized H_2O (10mg NHS-ester/100[□]µl H_2O) and 5[□]µl were added to each sample. The reaction mixture was vortexed and left for 15 minutes at room temperature to react. The samples were acidified by adding 1[□]µl 10% TFA and purified through [□]µC_{18}ziptips (Millipore) according the instructions of the manufacturer. The sample was eluted directly on the MALDI-target with a saturated solution of alpha-cyano-4-hydroxycinnamic acid in 50% AcN:0.1%TFA and analyzed in reflectron positive mode and PSD mode positive mode using the Ettan™ MALDI-ToF.

Please amend the paragraph at page 34, lines 26-29, as follows:

dPSD of NHS-ester derivatized tryptic digests of proteins from E-coli

Preparation of low speed supernatant of *Escherichia coli- Escherichia coli* (E-coli), (40 [□]µg stain B, ATCC 11303) was put in 20 ml reducing buffer containing 8M urea/4 % chaps, 2% 3-10 pharmalyt, 65 mM DTT. The cells were disrupted by sonication (7 x 20s

Please amend the paragraph at page 35, lines 22-29, as follows:

N-terminal derivatization: The samples were reconstituted in 20[□]µl deionized H_20. One [□]µl (20%) of each sample was mixed 1:1 with alpha cyano matrix solution and analysed in reflectrone positive mode using the Ettan™ MALDI-ToF. To the remaining 19[□]µl of each sample, 1 [□]µl DIEA and 5 [□]µl sulfopropionic NHS-ester solution, 10 mg/100 [□]µl were added. The samples were thoroughly mixed by pipeting and left to react for 15 minutes at room temperature. TFA (1 [□]µl, 10%) was added to each sample and purified through [□]µC_{18}ziptips (Millipore). The samples were eluted directly on the